THE PROCESS OF FERTILIZATION IN THE SPINY LOBSTER *JASUS LALANDEI* (H. Milne-Edwards)

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SUMMARY

The mechanism of copulation in *Jasus lalandei* (H. Milne-Edwards) is discussed by comparing the reproductive organs with those of *Panulirus cygnus* (George). The structure of the fifth pereiopods and thoracic sterna of the female *J. lalandei* indicates that the deposition of external spermatophores is unlikely. The male genital apertures of *J. lalandei* are also not well adapted for depositing external spermatophores as they are in *P. cygnus*. The introduction of internal spermatophores is therefore the alternative.

An hypothesis is put forward, describing a potential intromittent organ in male *J. lalandei* to support the assumption of internal fertilization.

INTRODUCTION

The mechanism of fertilization has been described for several species of *Panulirus; Panulirus interruptus* (Randall), Allen (1916), Lindberg (1955); *Panulirus penicillatus* (Oliver), Matthews (1951); *Panulirus cygnus* (George); Sheard (1949), George (1957); *Panulirus argus* (Latreille), Walton Smith (1959). In each case fertilization occurs in the same manner. The male deposits a putty-like spermatophore on the sternum of the female, posterior to the genital apertures, some time before the eggs are released. The spermatophore, initially soft and light in colour, hardens and turns black. Eggs are said to be fertilized externally by sperm released from the spermatophore by the chela of the fifth legs of the female. However, the method of copulation has not been verified, nor is it known with certainty at what stage of moult copulation occurs.

Von Bonde (1936) described the act of mating for *Jasus lalandei* (H. Milne-Edwards) from observations made on captive animals. He found that the male turns a newly moulted female on her back so that their sterna are closely apposed. His conclusion that fertilization is internal is based on the following statement, that "the spermatophores are extruded and appear to make their way through the female genital apertures and so into the oviducts where fertilization takes place at their upper ends". Since no mention is made of actually finding spermatophores in the oviducts or failing to find them externally it must be taken that the above statement is an assumption.

Initial observation of external genitalia during this present investigation indicated that internal fertilization was difficult mechanically due to their relative positions and difference in size between the sexes. Copulation was not observed during observation of captive animals over three years, precluding direct description. The problem was therefore approached indirectly by comparing the external genitalia of *P. cygnus* from which the process of fertilization is known, with those of *J. lalandei*. From this comparison it was hoped that an hypothesis for the method of fertilization, based on more than assumption, could be erected.

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The Female

(a) *The Fifth Walking Leg*:—The fifth walking legs of *P. cygnus* and *J. lalandei* are compared in Fig. 1. In *P. cygnus* a short, stout arm projects laterally from the base of the dactylopodite, which is capable of closing against a stout extension of the propodite. The dactylopodite therefore forms a strong chela capable of pinching as well as scratching. This chela is used to break the spermatophore and then gouge it open to release sperm. In *J. lalandei* the dactylopodite has no lateral arm and is similar to those of the other walking legs. A spine projects from the distal end of the propodite, which is apposed to the dactylopodite forming a chela. The spine of the propodite is much smaller than the dactylopodite and is attached by a thick membrane of chitin. The chela is therefore not very strong as the spine of the propodite does not form a solid base to the dactylopodite. Such a chela is not suited for pinching or breaking and is probably used to comb and clean the ovigerous setae of the pleopods.

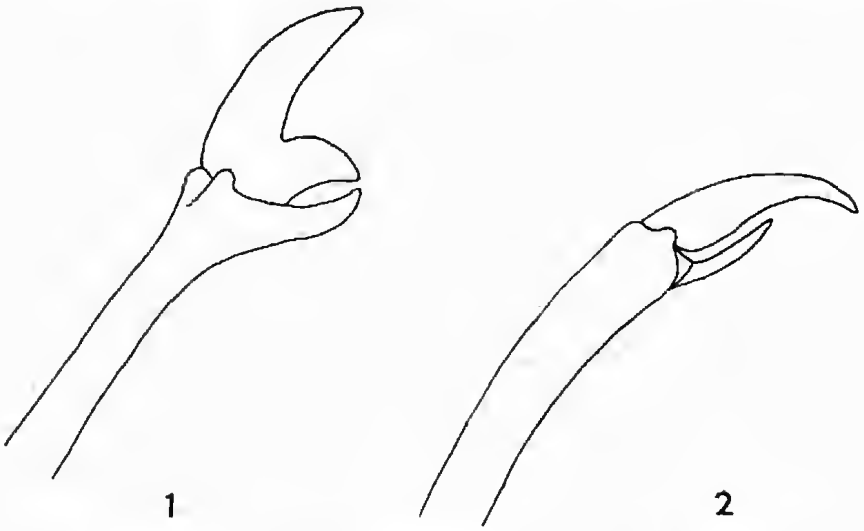

Fig. 1. The fifth chelate pereiopods of (1) *P. cygnus* and (2) *J. lalandei*.

(b) *The Last Two Thoracic Sterna*:—The last two thoracic sterna of *P. cygnus* have a smooth hairless area, presumably for the reception of spermatophores. This area is covered by tufts of short hairs in *J. lalandei* indicating that deposition of an external spermatophore is unlikely.

(c) *The Genital Apertures*:—Both species have similar genital apertures. The rim of the aperture is raised and circular in shape. The actual opening is situated on the inner side and extends as a crescent-like slit around half of the circumference. The remaining area inside the rim is filled with a chitinous membrane, which can be inverted to form a circular opening. This membrane is quite soft and easily inverted in *J. lalandei*, but is inverted with difficulty in *P. cygnus*. The diameter of apertures in mature animals is 2 to 3 mm in *J. lalandei* and 3 to 4 mm in *P. cygnus*.

The Male

The Genital Apertures (Fig. 2):—The genital aperture of *P. cygnus* has the form of an oval saucer. The actual opening to the vas deferens is slit-like and situated on the inner side of the saucer. The remaining area inside the saucer is filled with a chitinous membrane in the form of a loosely coiled tube ending in a spine. The spine is free and the tube is capable of erection. Normally, the tube is coiled so that the spine effectively closes the aperture. The tube is muscular and is probably capable of autonomous movement. Its probable function is to direct the placement of the spermatophore. Movement of the fifth legs moves the aperture in an arc, accounting in part for the bilateral symmetry of the spermatophore. Apertures of mature animals may be more than 12 mm in diameter.

The genital aperture of *J. lalandei* is much smaller than that of *P. cygnus*, being as small as 3 mm in diameter at first maturity. The shape is similar to the female aperture, the actual opening extending in an arc around the inner rim. The chitinous membrane filling the remaining area is folded and shaped to form a tongue-like flap, which normally closes the opening. It is unlikely that such an aperture could extrude a spermatophore similar to that of *P. cygnus*.

It can be seen from comparison of external genitalia that the chelate fifth legs of female *J. lalandei* are poorly adapted to break open external spermatophores. In fact, it is unlikely that external spermatophores could be attached successfully to the sterna of female *J. lalandei*. The soft nature of the female aperture in *J. lalandei* also indicates the possibility of introducing a spermatophore internally.

The large male genital apertures in *P. cygnus* would allow large quantities of spermatophoric material to be extruded. Their construction also allows the spermatophore to be directed over a relatively large area reducing its thickness. A thin spermatophore would be gouged more efficiently than a thick one, with better release of sperm. It is doubtful whether the smaller size of the male genital aperture in *J. lalandei* would allow the large amount of material necessary to form an external spermatophore to be extruded.

Most aspects of the external anatomy of *J. lalandei* indicate poor adaptation for external fertilization. If fertilization is internal, the absence of a long intromittent organ and the small size of the apertures introduces the problem of how males can locate the female aperture for efficient transfer of spermatophores. In an attempt to answer this question, a detailed examination of the male genital aperture was made.

It has been stated that the tongue-like flap of the male genital aperture normally closes the aperture. However, this flap is capable of erection and may project more than 5 mm in large males. This observation introduced the possibility that the flap of male genital apertures could be used to locate female apertures. The validity of this suggestion appeared to lie in the mechanism of erection. The fact that most flaps were observed in the distended condition during breeding seasons indicated they were connected functionally with copulation.

The structure of the male genital aperture was therefore studied from transverse sections. Several male genital apertures were excised with some underlying muscle and a short length of vas deferens, and fixed in Gilson's fluid. The acetic acid of this fixative decalcified the skeleton, which was softened

further with 8 per cent phenol in 75 per cent methyl alcohol. After embedding in paraffin wax, m.p. 58°C., serial transverse sections 15μ thick were cut. Sections were stained with Delafield's haematoxylin (Harris modification) and eosin.

The flap had no muscle-attachment, eliminating the possibility of erection through contraction of muscles. The underlying tissues contained large blood spaces, suggesting the flap was distended by an increase in blood-pressure. It has been suggested by Von Bonde (1936), that mating occurs a few weeks following moulting by the male. Since increase in size at moult is caused primarily by an increase in tissue-fluid it is possible that erection of the flap is a consequence of moulting. Von Bonde also stated that the female moulted a few hours prior to mating. At this stage the skeleton is very soft and the

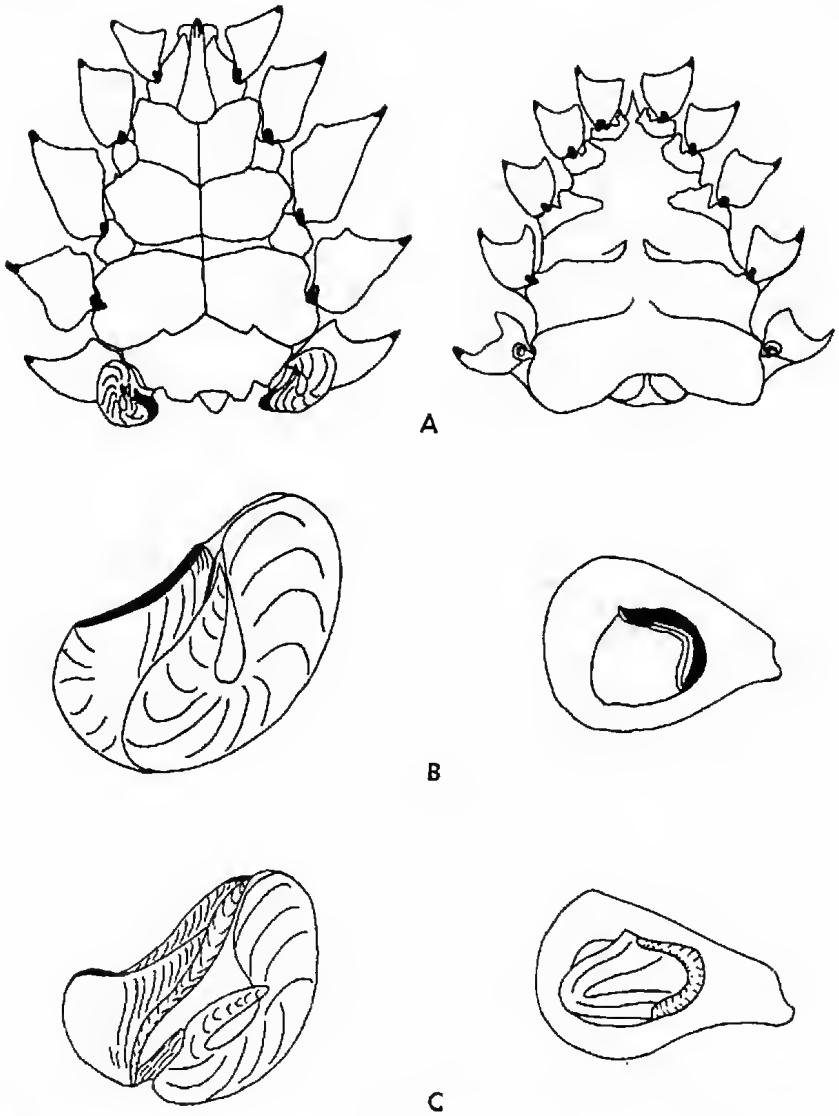

Fig. 2. The male genital apertures of *P. cygnus* and *J. lalandei*. A. Genital atria *in situ*. B. Genital flaps closed. C. Genital flaps open.

chitinous membrane of the female aperture would be inverted easily. This would therefore be the best time for the male to locate the female apertures and maintain its position by insertion of the genital flaps.

Examination of the oviduct failed to reveal a dilatation or sac that could be used as a seminal vesicle. Without such a vesicle it was difficult to see how fertilization could occur in the oviduct. Even allowing for stretching of the oviduct it would be much smaller than the corresponding vas deferens and it is doubtful whether it could hold the same amount of spermatophoric material. It was also difficult to see how sufficient of the spermatophore was retained to fertilize all eggs after ovulation had begun.

It has been stated previously (Fielder, 1964) that the oviduct is lined with high columnar epithelium, which is folded to form villi. In many cases adjacent villi formed sac-like channels. Apart from secreting a lubricating fluid or contributing to the egg-shell, it is difficult to see the significance of villi in the oviduct. One other possible function of villi would be to retain sperm, which would fertilize the eggs as they passed down the oviduct. This could occur only if some of the matrix of the spermatophore was removed and sperm concentrated between the villi of the oviduct. A final comparison was made between the structure of the sperm-mass and vas deferens of *J. lalandei* and *P. pencillatus*. The purpose of this comparison was to determine whether the spermatophore of *J. lalandei* was more likely to be deposited externally or internally. The vas deferens of each species has been described earlier, *J. lalandei* (Fielder, 1964) and *P. pencillatus* (Matthews, 1951). The glands of the proximal vas deferens of *P. pencillatus* secrete a crystalline material, which surrounds the sperm-mass. This walled sperm-mass continues into the large distal portion of the vas deferens. Here it becomes convoluted and embedded in a matrix secreted by a large glandular "typhlosole". Sections through the distal vas deferens show sperm concentrated into a strand contained within the granular spermatophoric wall, the whole embedded in a non-cellular matrix.

The proximal vas deferens of *J. lalandei* does not secrete a granular wall around the sperm-mass, but appears to initiate secretion of a fluid matrix. A distinct strand of sperm is therefore not formed. The resultant spermatophore appearing in the distal vas deferens consists of clumps of sperm embedded in the fluid matrix. A very thin crystalline wall appears to surround the matrix.

Matthews (1951) described the spermatophoric mass of *P. pencillatus* as being putty-like on extrusion. At a similar stage the spermatophoric mass of *J. lalandei* is a sticky, jelly-like mass, which remains discreet in sea-water. It is reasonably fluid and could possibly be introduced into the oviduct. Absence of a crystalline wall around the sperm-mass would allow release of sperm on disintegration of the matrix. Such disintegration of the matrix in the oviduct would allow sperm to be stored between the villi in the oviduct until needed.

It appears on morphological grounds then, that Von Bonde's assumption was correct and that fertilization in *J. lalandei* is internal. Observations of captive animals also indicated that moulting of the female is a prerequisite for mating. Four females moulted between August and October. Although males were present, mating was never observed. In each case, however, the female died within two weeks of moulting without appreciable hardening of the exoskeleton. Post-mortem examination showed that the ovaries were ripe. No sperm were detected in the oviduct or in the ovary. No external spermatophore had been deposited. It must therefore be assumed that mating had not occurred.

The fact that the four animals died without spawning may indicate that mating is a necessary stimulus for spawning and failure to spawn may prove fatal. Lindberg (1955) states that: "It is not known whether eggs will be extruded in the absence of a sperm case, or without mating activity, but it is perhaps significant that only fertilized eggs attach to the swimmerets. The presence, in females not bearing sperm cases, of ripe ovaries late in the breeding season may indicate, in fact, that egg extrusion does not occur in the absence of mating."

DISCUSSION

In the absence of critical observation of mating and extrusion of eggs, three factors appear necessary for successful spawning. These are (1) moulting precedes mating, (2) spermatophores are introduced in the oviducts where fertilization occurs, (3) mating is probably a prerequisite of spawning.

It is unlikely that both genital apertures of the male and female would often coincide during mating. It is also unlikely that eggs are extruded from one genital aperture only. If fertilization does occur in the oviduct, sperm must be present in both oviducts if fertilization is to be complete. Further work is required to determine whether a male is able to control extrusion of spermatophores or whether some sperm is lost by release from both apertures when one does not coincide with a female aperture.

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